Claim 25(NEW). The method of claim 22, wherein said diagnosis or monitoring is carried out on multiple samples such that at least one analysis is carried out on a first sample and at least another analysis is carried out on a second sample.

A.1 cancld

Claim 26(NEW). The method of claim 22, wherein said first and second samples are obtained at different time periods.

REMARKS/ARGUMENTS

Claims 22-26 remain in this application. Claims 1-21 have been cancelled. Claims 22-26 have been added.

In response to the Office Action of March 13, 2002, Applicant requests re-examination and reconsideration of this application for patent pursuant to 35 U.S.C. 132.

Rejections under 35 USC 112

Claims 1-21 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The reasons for rejection are as follows:

Claim 1, line 10 the recitation "associated" is vague.

It is unclear what kind of association applicant is referring to.

Claim 1, line 10, "contacting" is vague. How does this contacting differ from other contacting? There are different methods of ELISA testing and thus contacting may be different.

Claim 1, line 16 the recitation "achieved" is not a positive limitation and is vague. It is unclear what it encompasses. Is the diagnosis made or not?

Claim 4, line 2 the recitation "the signal to noise ratio" there is insufficient antecedent basis for this limitation.

Claim 4, line 3 "contacting said sample" is vague. Does this contacting include the compound recited in claim 4, line f .

Claim 4, line 4 the recitation "high specific affinity" is vague. It is unclear what is considered to be a high specific affinity.

Claim 11, lines 9,11,12, 13, 14, 15 and 18, the recitation "biomolecule" is unduly broad and encompasses more than the specification could possibly support. For example on page 34 in the specification the only disclosure of the biomolecule used in the kit appears in lines 20 and 21 which exemplifies the use of BMP and anti-MBP-IgG and anti-MBP IgM and no other biomolecules are disclosed in the specification. See also deficiencies found in claims 13-18.

Claim 11, line 16 "one analysis determinative" is vague.

How is this analysis performed?

Claim 11, lines 19 and 20 "provides a means for diagnosing or monitoring disease state" is vague. How does this provide a means?

Rejections under 35 USC 102(e)

Claims 1 and 2 are rejected under 35 U.S.C. 102(e) as being anticipated by Bloch et al (US Patent 6,183,988).

Bloch et al disclose a diagnostic method for multiple sclerosis which comprises obtaining biological samples from a mammalian body fluid such as sera, plasma, CSF, or saliva. Bloch et al disclose contacting the sample with at least one protein (Sp140) and the contacting is by an enzyme-linked immunosorbent assay (col 22 line 52 - col 23, line 22).

Bloch et al disclose detecting the binding of autoantibodies in the serum sample (col 4, lines 1-21). Bloch et al also disclose comparing the level of at least one autoantibody in the first biological sample is measured or estimated and compared to that in a standard taken from an individual not having the autoimmune disease (col 22, lines 37-51). Bloch et al also disclose that at least one protein (Sp140) immobilized on a solid support such as an immunosorbent (col 23, lines 1-8). Bloch et al also disclose the use of a second antibody used for detection. Bloch et al disclose that the identity of this second antibody will depend upon the identity of the mammal for which the biological sample to be tested is derived; for example, if it is a human serum sample, the second antibody will be an anti-human antibody (col 23, lines 17-22).

Rejection under 35 USC 103(a)

Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bloch et al (US Patent 6,183,988) in view of Elrod et al (US Patent 5,861,264). See above for the teachings of Bloch et al.

Bloch et al differ from the instant invention in failing to teach the protein associated with multiple sclerosis as being myelin basic protein.

Elrod et al are cited as disclosing that multiple sclerosis has been associated with the presence of autoantibodies against myelin basic protein and that multiple sclerosis is characterized by the presence of autoantibodies against this normal endogenous body constituent.

It is the Examiner's position that it would have been obvious to one of ordinary skill in the art to substitute myelin basic protein as taught by Elrod et al for the Sp140 protein of Bloch et al because Elrod et al shows that multiple sclerosis has been associated with the presence of autoantibodies against myelin basic protein and that multiple sclerosis is characterized by the presence of autoantibodies against this normal endogenous body constituent.

Claim 4 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bloch et al in view of White et al (US Patent 5,821,064). See above for teachings of Bloch et al.

Bloch et al differ from the instant invention in failing to disclose mixing the sample with at least one compound effective to optimize the signal to noise ratio.

White et al disclose the addition of heparin to an ELISA assay. The addition of this heparin allows for reducing backgrounds, enhancing assay signals and increasing assay sensitivity (col 7, line 60 - col 8, line 8, see also col 4, lines 11-14).

It is the Examiner's position that it would have been obvious to one of ordinary skill in the art to incorporate the use of heparin as taught by White et al into the method of Bloch et al because White et al shows that the addition of heparin allows for reducing backgrounds, enhancing assay signals and increasing assay sensitivity.

Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bloch et al (6,183,988) in view of White et al (US Patent 5,821,064) as applied to claims 1, 2, and 4 above, and further in view of Lihme et al (US Patent 6,221,634).
See above for teachings of Bloch et al and White et al.

Bloch et al and White et al differ from the instant invention in failing to teach the signal generating system is a tetramethylbenzidine substrate.

Lihme et al (US Patent 6,221,624) disclose a tetramethylbenzidine substrate. This substrate is especially suitable for enzyme assays such as enzyme-linked - immunosorbent-assays (ELISA), e.g. horseradish peroxide (HRP) is used, and this substrate is storage stable for more than 12 months.

It is the Examiner's position that it would have been

obvious to one of ordinary skill in the art to incorporate the use of a tetramethylbenzidine substrate as taught by Lihme et al into the method of Bloch et al because Lihme et al shows that this substrate is especially suitable for enzyme assays such as enzyme-linked immunosorbent-assays, and this substrate is storage stable for more than 12 months.

Claims 6, 8 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bloch et al (US Patent 6,183,988) in view of White et al (US Patent 5,821,064) as applied to claims 1, 2, and 4 above, and further in view of Voumbourakis et al (Detection of anti-MBP in the serum of patients with multiple sclerosis, Deltion Ellenikes Mikrobioloqikes Etaireias, (1992) Abstract Only). See above for teachings of Bloch et al and White et al. Bloch et al and White et al differ from the instant invention in failing to teach the autoantibody is anti-MBP IgG and anti-MBP IgM.

Voumbourakis et al disclose determining anti-MBP of the IgG and IgM isotypes. The aim of this study was to investigate anti-MBP in the serum of patients with M.S. since the occurrence of these antibodies in subjects with multiple sclerosis is controversial.

It is the Examiner's position that it would have been obvious to one of ordinary skill in the art determine anti-MBP IgG and IgM as taught by Voumbourakis et al for the method of Bloch et al because Voumbourakis et al teach that the pathogenisis of multiple sclerosis involves antibodies directed against myelin

basic protein and that the aim of the study was to investigate anti-MBP in the serum of patients with M.S. since the occurrence of these antibodies in subjects with M.S. is controversial.

Claims 7 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bloch et al in view of White et al, and Voumbourakis et al as applied to claims 1,2, 4, 6, and 8 above, and further in view of Targoff et al (US 6,160,107).
See above for teachings of Bloch et al., White et al., and Voumbourakis et al. Bloch et al differ from the instant invention in failing to teach the antibody composition being comprised of anti-human IgG conjugated to horseradish peroxidase and also fails to teach the antibody composition comprised of anti-human IgM conjugated to horseradish peroxidase.

Targoff et al (US Patent 6,160,107) disclose that in order to detect human autoantibodies, a goat anti-human immunoglobulin antibody may be used in a form in which it is conjugated to horse-radish peroxidase. The use of these anti-human antibodies conjugated to horse-radish peroxidase provides methods for detecting autoantibodies found in the sera of individuals (col 4, lines 60-63).

It is the Examiner's position that it would have been obvious to one of ordinary skill in the art to incorporate the use of anti-human antibodies conjugated to horse-radish peroxidase as taught by Targoff et al into the method of Bloch et al because Targoff et al shows that the use of these anti-human antibodies conjugated to horse-radish peroxidase provides methods for

detecting autoantibodies found in the sera of individuals.

With respect to the anti-human antibodies being IgG or IgM as recited in the instant claims. The IgG and IgM antibodies are response dependent immunoglobulins and it would have been obvious to one of ordinary skill in the art to select the appropriate immunoglobulin for optimization of the method. Also the optimum anti-human antibody as recited in the claims can be determined by routine experimentation and thus would have been obvious to one of ordinary skill in the art. It has long been settled to be no more than routine experimentation for one of ordinary skill in the art to discover an optimum value of a result effective variable. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum of workable ranges by routine experimentation." Application of Aller, 220 F.2d 454,456, 105 USPQ 233, 235-236 (C.C.P.A. 1955). "No invention is involved in discovering optimum ranges of a process by routine experimentation ." Id. At 458,105 USPQ at 236-237. The "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." Application of Boesch, 617 F.2d 272,276, 205 USPQ 215, 218-219 (C.C.P.A. 1980).

Claims 11-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bloch et al, in view of Elrod et al, White et al, Lihme et al, Voumbourakis et al and Targoff et al as applied to claims 1-10 above, and further in view of Boguslaski et al (US Patent 5,420,016).

See above for teachings of Bloch et al, Elrod et al, White et al,

Lihme et al, and Voumbourakis et al.

Bloch et al differ from the instant invention in failing to package the components into a kit. Boguslaski et al disclose assembling various system components into a test kit. By assembling these components into test kits,
it makes it more convenient and facile for the test operator (col 7, lines 8-11).

It is the Examiner's position that it would have been obvious to one of ordinary skill in the art at the time the invention was made to assemble the various reagents into kits such as taught by Boguslaski et al because Boguslaski shows that test kits make it more convenient and facile for the test operator.

REMARKS

In response to the Examiner's rejections claims 1-21 have been canceled and replaced with new claims 22- .

The references have been carefully considered, and a translation of the article to Voumvorakis et al is herein provided.

The claims are now limited to the determination of MBP autoantibody using an ELISA method characterized by its use of heparin sulfate.

The general protocol of the ELISA method by Voumvourakis et al. contains many similarities to all ELISA based assays. The essential difference between the ELISA assay of Voumvorakis and that of the instant invention is as

follows. Voumvourakis et al. use n histone to block binding sites on the ELISA plate. Histone is a basic protein with a charge similar to myelin basic protein. It does not bind to myelin basic protein. It blocks binding sites on the plates only. The instant inventor's experimented with the use of histone, but found that it was not helpful in reducing the background.

The instant method uses heparin sulfate, a polymeric carbohydrate which is negatively charged. It binds to positively charged myelin basic protein to block antibody binding sites on the antigen. This phenomena was found to successfully decreased the background and permitted a difference to be discerned between normal and MS samples.

The references, taken singly or in any combination, fail to teach or suggest the use of an ELISA assay which utilizes heparin sulphate bound to non-specific binding sites on myelin basic protein, thereby permitting the an assay whose specificity is due to binding of serum antibodies to specific binding sites on myelin basic protein.

SUMMARY

In light of the foregoing remarks and amendment to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,

Ferris H. Lander

Ferris H. Lander
Registration # 43,377

McHale & Slavin, P.A.
4440 PGA Blvd., Suite 402
Palm Beach Gardens, FL 33402
(561) 625-6575 (Voice)
(561) 625-6572 (Fax)

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Acta Microbiologica Hellenica (1992) 37, 666 – 672

Detection of Anti-MBP in serum of patients with multiple sclerosis

K. VOUMVOURAKIS, I. SEGDITSAS, A. ROMPOS, M. KONSTANTOULAKIS, K. PAPAGEORGIOU

Summary

Evidence exists that the immune system takes part in the pathogenesis of multiple sclerosis (MS), by immune mechanisms against antigens of the nerve tissue, and possibly through the production of antibodies against the myelin basic protein (anti-MBP).
The purpose of this study is to track anti-MBP in the serum of MS patients, given that the research results for these types of antibodies are contradictory. Using the immuno-enzymatic technique ELISA with Pork MBP, we searched for anti-MBP, IgG, IgA, and IgM isotypes in the serum of 20 MS patients and 44 patients with nervous system disorders or lumbago, who were the control group. From the 20 patients, 12 (60%) presented increased anti-MBP, in above average values from the control group plus 3 SD. Six of the 12 patients showed anti-MBP of the IgG type, 2 of the IgA type, 2 of the IgG type and 2 anti-MBP of all three isotypes.

From the Investigative Laboratory of the Neurological Clinic of the University of Athens and the Microbiological Laboratory of Aiginitio Hospital

Anti-MBP tracking in serum of patients with multiple sclerosis667

Introduction

Multiple sclerosis (MS) is an inflammatory disease that affects exclusively the white matter of the central nervous system (CNS). Even though the disease is considered to be multifactorial, evidence exists that the immune system is one of the main factors that contribute to its pathogenesis. This view has been reinforced considerably by the discovery of anti nerve tissue antibodies, and mainly anti Myelin Basic Protein (MBP).

According to the studies of Cuzner & Davison[1], MBP is the antigen that is responsible in allergic experimental cerebral myelitis (PPSP) suspected in MS pathogenesis. In 1974 anti-MBP antibodies were found in serum and in the cerebrospinal fluid (CSF) in laboratory animals with PPSP[23], and in 1976 Johnson and Associates[4] discovered such antibodies in the CSF of MS patients.

Different studies for the determination of antibodies in serum and CSF in MS patients followed, however the results were contradictory. Certain researchers stated the finding of anti-MBP in low levels in patients with MS[5], and others discovered the existence of such anti-MBP[6]. In 1986, Garcia - Merino et. al.[7] discovered antibodies in serum and in CSF in significant levels and recently, in 1989, Warren and Carz[8] found anti-MBP in the CSF of patients in a rather significant percentage (89%).

In recent years, in order to discover these antibodies, the immuno-enzymatic method ELISA has been used because this method is considered simpler in execution and because it requires small amounts of material providing more stable reactions[9].

Materials and Methodology

Twenty MS patients were studied: 8 men and 12 women, ages ranging from 16 – 49. The median age for disease onset was 28. All patients were treated at the Neurological Clinic of the University of Athens and were in advanced stages of the disease. The patients underwent complete clinical and laboratory examinations, and, according to the prevailing criteria[10], 18 of these patients were suffering from proven disease and 2 from a suspected form of the disease.

Of our patients, 10 never had received therapy – mainly due to the recent onset of the disease – and the remaining 10 were given corticoids, but the regimen was interrupted at least 6 months before the study.

The anti-MBP tracking was done using the immuno enzymatic method ELISA in MS patients serum that was diluted 1/50.

As an antigen Pork Myelin Basic Protein (PMBP) was used in a concentration of 5 μg/ml. Three antibody isotypes were searched and discovered to be immunoglobulin conjugates with peroxidase enzyme of the IgG, IgA, and IgM type in a 1/1000 solution. In order to avoid the non specific reactions with histones, which to a large extent prevent these types of reactions[1112]. O – Phenyl – Diamine (O-P-D) was used as a chromogenic substrate. The results were read by taking visual density in a filter of 492nm wavelength. 10 ml of blood was drawn from the patients. The blood then was centrifuged at 2000 rpm for 10 minutes. The top layer was then removed and was kept at –70°C until needed.
Additionally, 44 patients of ages similar to the patients were also examined. These patients were the control group. Of them, 24 showed symptoms of lumbago, and 20 mentioned physiological reasons. All were submitted to full clinical and laboratory examinations for pathological diseases, including those of the autoimmune system, which were negative.

Results

Of the 20 patients that were examined, 12 showed increased anti-MBP levels, in values that were always higher than the average of the healthy plus 3 SD. Specifically, six patients showed increased anti-MBP levels of the IgG type, 2 patients showed increased anti-MBP levels of the IgG & IgA type, and the remaining 2 patients showed increased anti-MBP levels of all three isotypes. (Table 1).
The statistical analysis of the results was made with the student –t– test and showed that only the patients with the IgG and IgA isotypes showed antibody levels of a statistically significant difference, when compared with the control group ($P<0,01$ and $P<0,05$, respectively). (Table 2).

TABLE 1
Patients with increased anti-MBP levels

ISOTYPE	NUMBER
IgG	6
IgA	2
IgG + IgA	2
IgG + IgA + IgM	2
TOTAL	12 (percentage 60%)

In summary, we note: 1) the generally high percentage (60%) of the patients with increased auto antibody

activity, Anti-MBP tracking in serum of patients with multiple sclerosis 669
2) the discovery of IgG and IgA anti-MBP in statistically significantly differences, when compared with the control group.

TABLE 2
Anti-MBP isotypes in the Patient study
[left side of Table] Optic density
[bottom of Table] Serum
[ILLEGIBLE]
[ILLEGIBLE]

Discussion

In our study, 12 patients in total who suffered from a proven form of MS showed increased auto antibody activity and the remaining 8 (6 with a proven and 2 with a suspected form of the disease) did not have antibodies. The existence of such antibodies in such a high percentage in MS patients is an indication of a possible systemic disease, a view that has been supported recently by Hafler and Weimer[13]. In fact it is this that the generalized malfunction of the immune system leads to the development of B-lymph cells, resulting in overproduction of antibodies[14], which, since possibly by passive transudation reach the CNS[3], partake in the procedure of demyelination.
Similar to our own study, a study was done by Newcome and Associates[15], who using the immuno-blotting method, determine in the serum taken from 49 MS patients antibodies of the IgM, IgG and IgA type in 94%,

54% and 47% respectively. However, these antibodies were antibodies against different types of the CNS proteins and specifically against tubulin, myelin basic protein, protein of the 69K neurofibril, gliadin fibrous acid protein – G F A P – glycoprotein conjugated with myelin – MAG –, Wolfgram protein.

Additionally, Garcia – Merino and Associates[7] determined through ELISA in serum and CSF, in 37 MS patients, anti-MBP of the IgG type, in an amount of 41% and 27% respectively, without reaching definitive conclusions for the location in which these antibodies are produced. In 1988, Blancher and Associates[14] determined increased composition of auto antibodies – among which was anti-MBP – within the CNS. Finally, recently, in 1989, Warren and Catz[3] discovered anti-MBP in CSF in MS patients in a comparatively very large percentage (89%).

In our study, the discovery of IgG and IgA antibodies with a similarly significant difference when compared to the control group, shows that perhaps in the pathogenesis of the disease more than one of the antibody isotypes are involved. This is in accordance with the findings of other autoimmune diseases, such as the systemic lupus erythematosus, where more than two antibody isotypes are found[16]. Additionally, recently, Olsson and Associates[17] reported the discovery of a large number of IgG type anti-MBP producing cells, and a smaller number of IgA type anti-MBP producing cells in CSF.

However, in order for one to derive clear conclusions regarding the location of production and the activity of the anti-MBP, the testing of larger numbers of MS patients with concurrent testing for anti-MBP in the serum and in the CSF is deemed necessary. Also, the combined evaluation of the hematocerebral block and the study of patients with other neurological diseases will aid in the full understanding of the biological value of these antibodies.

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